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Environmental Science

A STUDY ON MICROBIAL REDUCTION OF HEXAVALENT CHROMIUM IN INDUSTRIAL WASTEWATER

Dr. Ibrahem E. Omran*	Alexandria university, Institute of Graduate Studies and Research, Department of Environmental studiesAlexandria, Egypt. *Corresponding Author
Prof. Dr. Ahmed M. Attia	Alexandria university, Institute of Graduate Studies and Research, Department of Environmental studiesAlexandria, Egypt.

ABSTRACT Chromium occurs in each of the oxidation states from -2 to +6, but only the 0 (elemental), +2, +3 and +6 states are common. The trivalent and hexavalent oxidation states are important for human health. In the context of this research, the cell membrane is nearly impermeable to Cr^{+3} "has only approx. one thousand less of the toxicity of Cr^{+6}".

Objectives: Reducing Cr^{+6} to Cr^{+3} simplifies its removal from effluent and also reduces its toxicity and mobility.

Methods/Statistical analysis: The study recruits natural micro-organisms samples isolated from chromium contaminated effluents from coating factory and dyeing factory specify the area; the next part of the task was to increasing the tolerance of the micro-organisms in order to improve the microbial capability in removing toxic chromium from the effluent either by adsorbing on cell wall or reduction inside the cell. experimental was carried in mineral salt media for 24 hr. at 30°C and 120 rpm, 2ml of growth bacteria "from previous concentration experiment" was taken as inoculum, Bacterial growth indication was carried by measuring the turbidity (Optical Density "OD")

Findings: The study found that a combination of three micro-organisms: *Staphylococcus warneri*, *Pantoea vagans* and *P. ananatis* can remove up to 91.5% of Cr^{+6} concentration in the media, and tolerate Cr^{+6} concentration up to 880 ppm.

Application/Improvements: The study recommends the bioremediation approach as a solution for the chromium contamination.

KEYWORDS : Cr^{+3}, Cr^{+6}, Chromium, Mineral salt media, Microorganisms, bioremediation

INTRODUCTION

Chromium (atomic number 24, relative atomic mass 51.96) occurs in various oxidation states but only the trivalent and hexavalent oxidation states are the most important[1-3]. Chromium used in the metallurgical processing, Oxidation, and purification of chemical, production of pigment, dyes, Fungicides and wood preservation[4-5].

Hazard of Cr^{+6} is easily noticeable with microorganisms which affected Gram-negative bacteria (LD_{50} 1-12 mg/kg) than gram-positive bacteria[6-7], in Plants toxic at high concentrations (LC_{50} 30 – 60 mg/liter for 3 days)[8-10], in aquatic Organisms toxicity depending on species, it can be less toxic in warm water or with increasing pH or hardness[11-13], in animals the toxicity depends with route of entry into the body[14-15], in Human acute Toxic effect in adults(LD_{50} 50-70 mg/kg) by oral dose with clinical features of toxicity like vomiting, diarrhea, hemorrhagic diathesis and blood loss into the gastrointestinal tract causing cardiovascular shock[15-16]. Chronic Toxic effects on skin and mucous membranes "ulcers (corrosive reactions)"[17], on the lung "corrosion in the pulmonary tract"[17-19], on the kidney "hyaline and granular casts and red cells appearing in the urine"[20-22], on liver "loss of its architecture"[23-24], mutagenicity and Carcinogenicity effect[25-31].

The cell membrane is nearly impermeable to Cr^{+3}, thus Cr^{+3} has only approx. one thousand less of the toxicity of Cr^{+6}. Because the insolubility of Cr^{+3} facilitates its precipitation and removal, the biotransformation of Cr^{+6} to Cr^{+3} has been considered as an alternative process for treating Cr^{+6} contaminated waste[32-34] as shown in Figures 1-2. Thus, reducing Cr (VI) to Cr (III) simplifies its removal from effluent and also reduces its toxicity and mobility[35-36].

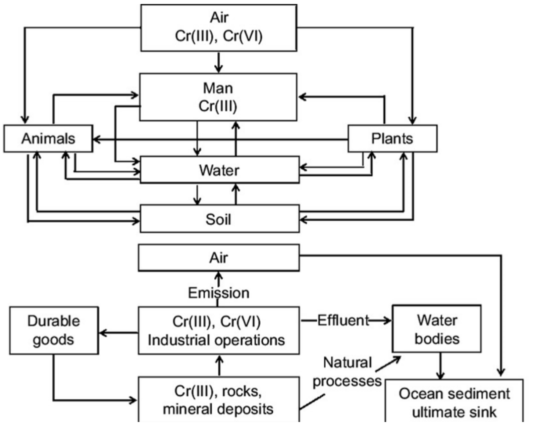

Figure 1.Chromium cycle

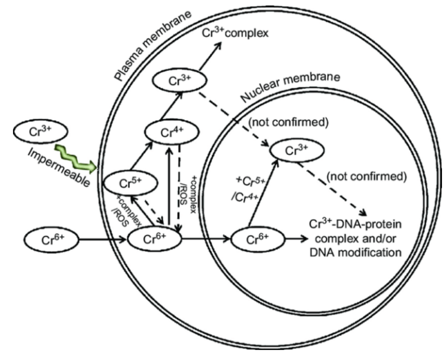

Figure 2. Schematic diagram of toxicity and mutagenicity of Cr+6, the intercellular Cr+6 reductants are frequently obligatory one electron reducers, which generate Cr+5 and large amount of ROS that causes the deleterious effect of Cr+6.

MATERIAL AND METHODS:

The study was carried out at Genetic Engineering and Biotechnology Research Institute (GEBRI), City for Scientific Research and Technology Applications (CSRTA), New Borg El-Arab city, Alexandria, Egypt. The study is based on bacterial isolates which were isolated from water and activated sludge samples[37-38] from sewage of painting company and textile dyeing company to measure its ability for bio-reduction of hexavalent chromium wastes[39-40]. These bacteria were isolated and then identified using molecular tools; the effect of a concentration on its removing ability was studied to increase its degradability, which is the main objective of the present study.

Samples: 13 Samples collected from Paints factory (7 water sample, 2 sludge, and 4 cotton swab) and 11 Samples collected from Tannery factory (6 water sample, 1 sludge and 4 cotton swab).

Hexavalent Chromium stock preparation: Chromium stock solution consisted of (g/1 de-ionized water): Potassium chromate (as Cr^{+6}) 186.74 g "K_2CrO_4", 1ml stock solution equivalent 50 mg Cr^{+6}.

Mineral salt medium (MSM) [41]**:** consist so K_2HPO_4 (2.4g), KH_2PO_4 (2g), $MgSO_4$ (0.01g), $CaCl_2$ (0.01g), NH_4NO_3 (0.1g) and complete it to liter with distilled H_2O then adjusted its pH to 7.2 ± 0.2 at 25°C.

Bacterial isolation and Screening: Chromium reduction bacteria were isolated from water and the effluent sludge by enrichment culture technique[42]. Two ml of sludge or 2 ml of contaminated water sample separately were inoculated to 50 ml sterile Luria Bertani broth media[43] (L.B.) in 250 ml Erlenmeyer flasks. Flasks were incubated for 24hr on a rotary shaker incubator under aerobic conditions at 30 °C and 120 rpm.

After activation on L.B. media, the activated bacteria (2ml as inoculum) is transferred as inoculum to mineral growth on salt medium (MSM) containing potassium chromate "K_2CrO_4" as hexavalent chromium source and glucose as carbon source.

Bacterium Growth: The designed experimental was carried in mineral salt media for 24 hr. at 30°C and 120 rpm[44-45]. Bacterial growth indication was carried by measuring the turbidity (Optical Density "OD") for mineral salt media as the direct proportion for microorganism's biomass.

Cell growth was determined by measuring the absorbance of an inoculated sample (Turbidity measurement) at 600 nm (A_{600}) on a spectrophotometer (UV-visible Cintra 40-GBC) using the fresh medium as blank, using turbidity as a direct indication for growth "ranged from 0.001 to 4 reported NTU"[46-47]. When specimen has ≥ 2 reported NTU, it qualified to use in the next higher concentration level experiment[48].

27 experiments were carried during this study "divided into 3 groups" to increase the tolerant and measured the consume percentage in each concentration as shown in Figure 3. Group one experiments "from 1 to 9" are for the activation of bacteria to tolerate the increasing concentration of hexavalent chromium "increase with minor amount", in Group two experiments "from 10 to 18" concentration increased by high amount "100 ppm equivalent Cr^{+6}" and in the last group of experiments "from 19 to 27" are specific for the specimen "2S" which survive in high concentration of Cr^{+6}; in this experiments Cr^{+6} concentration increased slightly until no bacterial growth observed "10 ppm increasing of equivalent Cr^{+6} concentration[49].

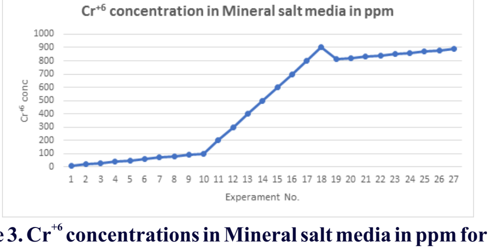

Figure 3. Cr^{+6} concentrations in Mineral salt media in ppm for each experiment

Measuring Chromium Content: For the estimation of residual chromium, centrifuged supernatants at 12,000RPM for 10 min. Supernatants were obtained after precipitation and proceed to extra purification from bacteria by filtrate by 0.2 µm sterile bacterial filters. The supernatants were taken as it is in higher concentration (≥100 µg/l) (measured by atomic absorption) and diluted to (1:15) by de-ionized water in case of concentration ranged from 10 – 90 µg/l (measured by spectrophotometric).

The supernatants were taken as it is in higher Chromium concentration (≥100 mg/l) and were measured by atomic absorption for the result of residue, removal and removal efficiency.

Result and discussion:

Regarding the bacterial uptake at concentration 300 mg Cr^{+6}/l (Figure 4), specimen 4 shows the highest consumption "281.4 ppm equivalent Cr^{+6}" then specimen 2S recorded in 2nd place with "274.5 ppm equivalent Cr^{+6}".

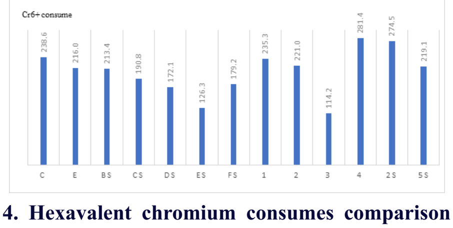

Figure 4. Hexavalent chromium consumes comparison at 300 mg/l, in MSM for 24 hr. at 30°C and 120 rpm.

Regarding the bacterial uptake at concentration 400 mg Cr^{+6}/l (Figure 5), specimen 4 shows the highest consumption"237.3 ppm equivalent Cr^{+6}" then specimen 2S come in 2nd place with "233.3 ppm equivalent Cr^{+6}".

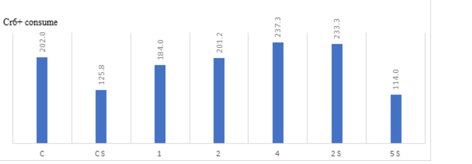

Figure 5. Hexavalent chromium consumes comparison at 400 mg/l, in MSM for 24 hr. at 30°C and 120 rpm.

Regarding the bacterial uptake at concentration 500 mg Cr^{+6}/l (Figure 6), specimen 4 shows the highest consumption "216.1 ppm equivalent Cr^{+6}" then specimen 2S come in 2nd place with "197.1 ppm equivalent Cr^{+6}".

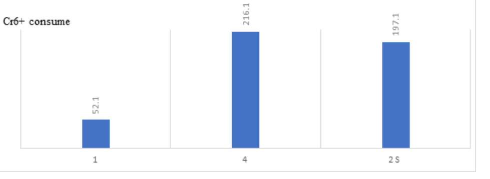

Figure 6. Hexavalent chromium consumes comparison at 500 mg/l, in MSM for 24 hr. at 30°C and 120 rpm.

Regarding the bacterial uptake at concentration 600 mg Cr^{+6}/l (Figure 7), specimen 2s show the highest consume "171.6 ppm equivalent Cr^{+6}".

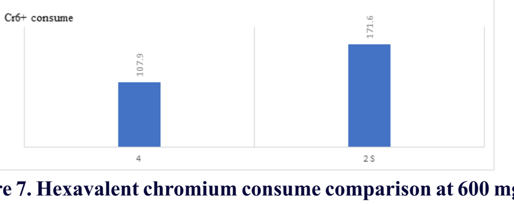

Figure 7. Hexavalent chromium consume comparison at 600 mg/l, in MSM for 24 hr. at 30°C and 120 rpm.

From the previous data, specimen "4" shows the highest removal of Cr^{+6} in the concentration 300, 400 and 500 mg Cr^{+6}/l but it decreases its removal capacity at 600 mg Cr^{+6}/l.on the other hand specimen "2s" shows the 2nd highest removal of Cr^{+6} in concentration 300, 400 and 500 mg Cr^{+6}/l it shows a more stable removal capacity at 600 mg Cr^{+6}/l than the specimen.

Specimen "4" shows the highest uptake at 300 mg Cr^{+6}/l (Figure 8) and decreases at 400 mg Cr^{+6}/l and continues in decreasing at 500 and 600 mg Cr^{+6}/l and didn't show growth at 700 mg Cr^{+6}/l.

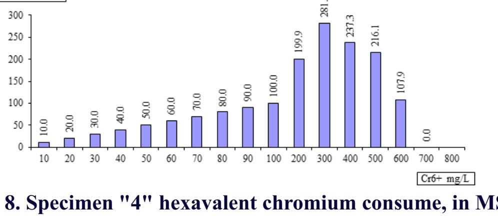

Figure 8. Specimen "4" hexavalent chromium consume, in MSM for 24 hr. at 30°C and 120 rpm.

Specimen "2s" shows the highest uptake (Figure 9) at 300 mg Cr^{+6}/l and decreases at 400 mg Cr^{+6}/l and continue to decrease from 500 to 880 mg Cr^{+6}/l and growth ceased at 890 mg Cr^{+6}/l.

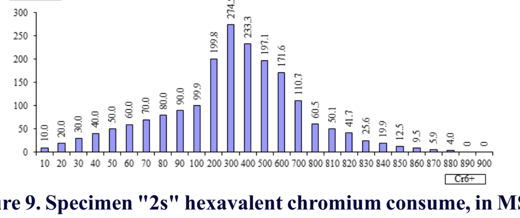

Figure 9. Specimen "2s" hexavalent chromium consume, in MSM for 24 hr. at 30°C and 120 rpm.

Comparison between Specimens at higher concentration of Hexavalent Chromium concentration: From the previous data specimen "4" shows the highest removal of Cr^{+6} in the concentration 300, 400 and 500 mg Cr^{+6}/l but it decreases its removal capacity at 600 mg Cr^{+6}/l. Though specimen "2s" recorded 2nd highest removal of Cr^{+6} in concentration 300, 400 and 500 mg Cr^{+6}/l it recorded better stable removal capacity at 600 mg Cr^{+6}/l than the specimen (name it).

Efficiency Study of Specimen "2s" (as highest Cr^{+6} resistant) at Different Concentration: This test is to study the specimen "2s" and its efficiency to remove Cr^{+6} in the all experiments and to identify the best growing concentration which give high efficiency of removal and good tolerance for hexavalent Chromium. It also to find tolerant higher concentration that can be expected in the industrial process.

According to [45], the OSM29 strain can remove Cr^{+6} in media concentration ranged from 600-1600 mg Cr^{+6}/l and can remove 100 mg Cr^{+6}/l in 24hr.

The best performance of specimen "2s" in the range between 300 – 500 mg Cr^{+6}/l with removal efficiency ranged from 91.5% to 58.3% (remove from 197 to 274 mg Cr^{+6}/l) in MSM for 24 hr. at 30°C and 120 rpm, and it can tolerate the Cr^{+6} until 880 mg Cr^{+6}/l with removal efficiency 0.5% in MSM for 24 hr. at 30°C and 120 rpm as shown in Figures 10-15.

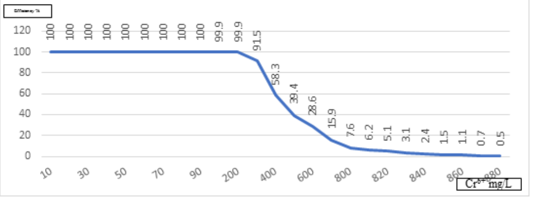

Figure 10. Specimen "2s" removal efficiency of hexavalent Chromium, in MSM for 24 hr. at 30°C and 120 rpm.

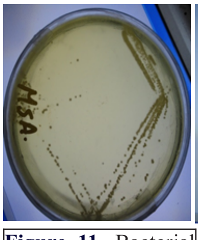

Figure 11. Bacterial Strain 1 (S1) grown in mannitol salt media for 24 hr. at 30°C

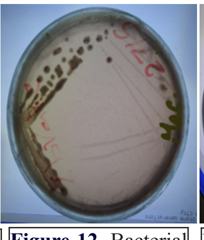

Figure 12. Bacterial Strain 2 (S2) grown in macconkey media for 24 hr. at 30°C.

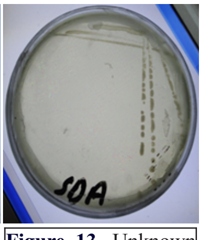

Figure 13. Unknown Strain 3 (S3) "bacterial or yeast" grown in Sabouraud Maltose salt media for 24 hr. at 30°C.

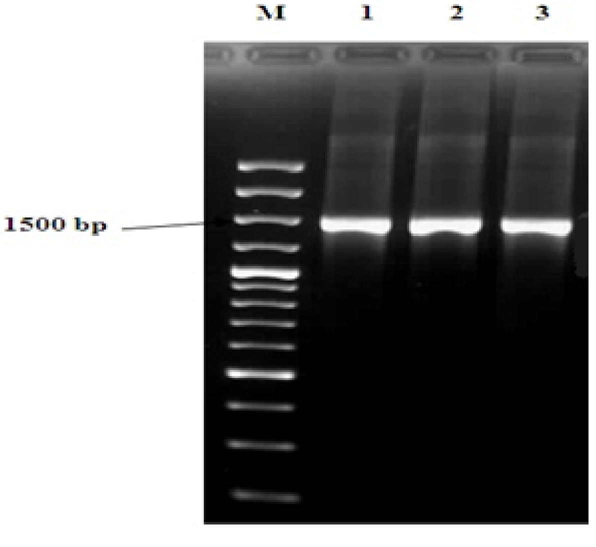

Figure 14. 16S rDNA PCR amplification product gel electrophoresis (MW=1500 bp). (M) is the marker, (1) Staphylococcus warneri, (2)Pantoea vagans, and (3)Pantoea ananatis

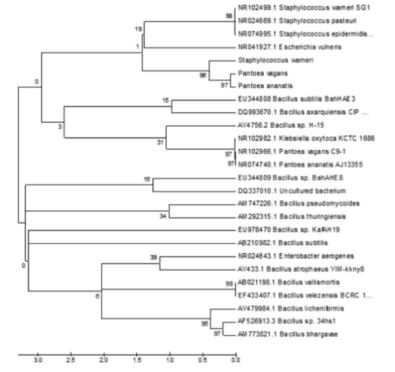

Figure 15. Phylogenetic tree of Staphylococcus warneri, Pantoea vagans, and Pantoea ananatis strains and their related genera have been linked based on partial 16S rDNA sequence comparisons

At concentration equal 600 ppm, specimen 2s remove 171.55 ppm of hexavalent chromium "equivalent 28.6% at concentration 700 ppm, specimen 2s remove 110.74 ppm of hexavalent chromium "equivalent 15.9%", which is higher than the OSM29 strain.

But at concentration equal 800 ppm, specimen "2s" remove 60.53 ppm of hexavalent chromium "equivalent 7.6%", which is lower than the OSM29 strain.

According to this, the specimen "2s" can remove the higher concentration of hexavalent Chromium than the OSM29 strain. but can't survive in higher concentrations as OSM29 strain.

Isolation and Screening of Bacteria: Bacterial colonies were picked and purified by repeated sub-culture to obtain pure isolates by selective agar media[50-55].

CONCLUSION

A mixture from *Staphylococcus warneri*, *Pantoea vagans* and *P. ananatis* can remove the higher concentration of hexavalent Chromium ranged from 28.6% to 15.9%at elevated concentration (600 – 700 ppm) and can survive till 890ppm of hexavalent chromium.

Recommendation.

The study recommended to use a mixture of *Staphylococcus warneri*, *P. vagans*, and *P. ananatis* strains to remove hexavalent Chromium Cr^{+6} from effluent at concentration ranged between 300 – 500 mg Cr^{+6}/l "efficiency ranged from 91.5% to 58.3%".

Further studies with substitute of bacterial strains may enhance the hexavalent chromium removal at higher concentrations.

The sludge produced from bacteria biomass can be used to retrieve Chromium, or can be buried in Hazardous waste landfills, if it wasn't economically sufficient.

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